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Hereditary Leiomyomatosis and Renal Cell Cancer: Clinical, Molecular, and Screening Features in a Cohort of 185 Affected Individuals

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Abstract

Background: Hereditary leiomyomatosis and renal cell cancer (HLRCC) is a tumour predisposition syndrome characterised by predisposition to cutaneous and uterine leiomyomata and renal cell carcinoma (RCC).
Objective: To define the clinical findings, molecular genetics, and prognosis in a cohort of 69 families with a fumarate hydratase (*FH*) pathogenic variant and/or clinical features of HLRCC.
Design, setting, and participants: Clinical and molecular findings were obtained for 185 individuals from 69 families from four UK regional genetics clinics.
Outcome measurements and statistical analysis: Ages at confirmed diagnoses, last dates of follow-up, and molecular results were attained for probands and relatives. To study the effect of potential ascertainment bias, phenotypes of probands and their affected relatives were compared.
Results and limitations: A germline *FH* variant (19 novel and 21 known, >50% missense variants) was identified in 68/69 probands and 90 relatives. Cutaneous leiomyomata occurred in 90/185 (48.6%) individuals (mean age 45.9 yr) and uterine leiomyomata in 33/107 (30.8%) females (mean age 35.0 yr). Of 185 individuals, 23 (12.4%) had a confirmed renal tumour, and histopathology where known ($n = 18$) was variable: seven clear cell RCCs, nine papillary RCCs (six of type 2), one collecting duct tumour, and one tumour with oncocytic cystic morphology.

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Mean age at symptomatic RCC diagnosis was 44.0 yr and median survival was 21.0 mo. Eighty-one individuals underwent 187 renal imaging surveillance scans; three stage 1 RCCs were detected. Mean survival of individuals diagnosed with stage 1/2 RCC was significantly longer than those diagnosed with stage 3/4 RCC (p = 0.0004).

Conclusions: Management of HLRCC is challenging as RCC occurs in a minority of cases but is highly aggressive. This large multicentre series has identified novel features and evidence that renal screening in HLRCC detects early-stage RCCs.

Patient summary: We show that hereditary leiomyomatosis and renal cell cancer is associated with a 21% lifetime risk of renal cell carcinoma (RCC; 95% confidence interval 8.2–37.1), and renal imaging screening detects early-stage RCC.

1. Introduction

Hereditary leiomyomatosis and renal cell cancer (HLRCC; #150800) is a tumour predisposition syndrome characterised by age-related predisposition to the development of cutaneous and uterine leiomyomata, and renal cell carcinoma (RCC) [1]. HLRCC arises from heterozygous germline alterations of fumarate hydratase (*FH*), which encodes the protein catalysing the conversion of fumarate to malate in the tricarboxylic acid cycle [2]. Homozygous germline mutations cause metabolic disorder and fumarase deficiency (FMRD; #606812); to date, there is no known difference between the *FH* mutations causing FMRD and HLRCC, and there are no discernable genotype-phenotype correlations for HLRCC.

HLRCC displays variable age-related penetrance with uterine leiomyomata and cutaneous leiomyomata reported to occur in up to 70% of affected individuals, whereas the risk of RCC is less clear but generally estimated to be 15–20% [1,3]. However, many previous studies have relied primarily on index case data to attain penetrance estimates.

The uterine leiomyomata and renal tumours seen in HLRCC typically have single or multinucleated nuclei with large inclusion-like eosinophilic or orangiophilic nucleoli surrounded by a clear halo [4,5]. These features have led to HLRCC, recently being classified as a distinct subtype of RCC [6], and are important to recognise as they may lead to new diagnoses of HLRCC. Immunohistochemistry, although not routinely available, to detect aberrant succination is highly specific for FH-deficient tumours, also aiding in the recognition of an underlying diagnosis of HLRCC [7].

HLRCC-associated RCC can be highly aggressive, and despite uncertainty and a paucity of evidence regarding both the magnitude of the risks of RCC and the efficacy of screening, an international consensus group recommended annual magnetic resonance imaging (MRI) renal imaging surveillance in at-risk individuals [1].

The prevalence of HLRCC has been estimated to be 1 in 200 000; therefore, it is challenging to identify the large numbers of families that might provide the information to enable better diagnosis and management of this rare disorder. Here, we describe the largest single cohort to date of HLRCC patients and family members including greater than two-thirds nonindex cases, detailing the clinical and molecular genetic features, renal screening, and RCC survival.

2. Patients and methods

2.1. Clinical studies

This study comprised patients and family members attending four NHS genetics clinics, with a likely diagnosis of HLRCC based on clinical diagnostic criteria [1] and/or molecular data [1]. Clinical and molecular information was collected from clinical records for research or service evaluation studies. Affected individuals were diagnosed by clinical examination and routine clinical investigations. All patients gave informed consent for genetic testing. This service evaluation study was approved by Manchester Foundation Trust, Birmingham Women's and Children's NHS Foundation Trust, St George's NHS Trust, and Nottingham University Hospitals NHS Trust. A subgroup of patients additionally consented for research studies. Clinically unaffected carriers of a known familial mutation were included. Family members testing negative for the familial mutation were excluded. No phenocopies were identified. Four families were previously reported [8–10]. Data for the age at diagnosis of RCC in HLRCC were compared with the previously published data for age at onset of RCC in von Hippel-Lindau (VHL) disease and familial nonsyndromic RCC [11,12].

For individuals in whom a clinical laboratory diagnostic report identified a germline *FH* variant, the previously unreported variants were retrospectively analysed based on the recently published criteria [13,14]. The review panel reached a consensus decision regarding variant classification according to the standard criteria [11] and contribution of the variant to clinical phenotype [15].

Renal imaging screening by MRI included T1, T2, fat sat, in and out of phase, diffusion and post-intravenous gadolinium images, and subtraction imaging. Images were acquired in axial and coronal planes, with slice thickness varying between 4 and 6 mm depending on the sequence.

Table 1 – Details of confirmed cutaneous and uterine leiomyomata occurring in HLRCC cohort.

Number with confirmed diagnosis			Means of confirmation			Age at diagnosis[a] (yr)				Cumulative prevalence to age 75 yr (%)		
Male	Female	Total	Histology	Clinical examination	Medical records	Mean age (SEM)	Median	Range	*N*	Male & female	Male	Female
Cutaneous leiomyomata (total cohort = 185)												
All												
32	58	90	54	36	3	45.9 (1.59)	45.5	18–70	82	78.0	74.9	79.9
Index												
13	31	44	35	8	1	42.7[b] (1.89)	43	18–43	39	94.7[c]	100.0	92.4
Nonindex												
19	22	46	19	25	2	48.7[b] (2.45)	47	22–79	43	68.0[c]	61.2	71.6

Number with confirmed diagnosis	Means of confirmation			Age at diagnosis[a] (yr)				Cumulative prevalence to age 75 yr (%)
	Histology	USS/MRI	Medical records	Mean age (SEM)	Median	Range	*N*	
Uterine leiomyomata (total female cohort = 107)								
All								
33	12	14	7	35.0 (1.59)	35	21–63	33	42.1
Index								
17	7	5	5	32.41[d] (2.02)	32	21–52	17	53.6[e]
Nonindex								
16	7	7	2	37.88[d] (2.90)	37	22–63	16	33.6[e]

HLRCC = hereditary leiomyomatosis and renal cell cancer; MRI = magnetic resonance imaging; SEM = standard error of the mean; USS = ultrasound scan.
[a] Where age was known.
[b] Mean age comparison of cutaneous leiomyomata, index with nonindex cases, $t = 1.89$, $p = 0.06$.
[c] Comparison of cumulative prevalence cutaneous leiomyomata, index with nonindex cases, across all ages, $\chi^2 = 12.14$, $p = 0.0005$.
[d] Mean age comparison (uterine leiomyomata), index with nonindex cases, $t = 1.56$, $p = 0.13$.
[e] Comparison cumulative prevalence uterine leiomyomata, index with nonindex cases, across all ages, $\chi^2 = 4.72$, $p = 0.0298$.

Renal cancers were staged as per the American Joint Committee Cancer Staging, seventh edition [16].

2.2. Molecular genetics studies

DNA was extracted from peripheral blood by standard methods and laboratory analysis undertaken by Sanger and/or next-generation sequencing according to the relevant protocols in the diagnostic laboratory (further details are available on request).

2.3. Statistical analyses

Statistical analysis was undertaken using Graphpad Quick Calcs, Graphpad Prism version 7, and SPSS version 20. Statistical significance was considered at 5%.

3. Results

3.1. Previous studies of HLRCC

We documented previous clinical studies of HLRCC (Supplementary Table 1). All had a smaller cohort number than that currently reported, with the larger studies having a high proportion of index cases.

3.2. Clinical features

There were 185 individuals (107 female and 78 male patients) from 69 families with a clinical and/or molecular diagnosis of HLRCC. The 69 families comprised (1) 57 new index case presentations (45 with cutaneous lesions, seven with a personal or family history of RCC, and five with a personal history of uterine fibroids), (2) 11 families with a diagnosis of HLRCC had been made in another centre, and (3) a single family with confirmed biallelic *FH* inactivation causing fumarase deficiency [8]. Mean age at diagnosis of HLRCC in 54 index cases (three ages unknown) was 45.2 yr (median = 46.5 yr, range = 17–70 yr, standard error of the mean [SEM] = 1.6).. Considering the non-index case presentations, there were 77 first-, 31 second-, and 14 third-degree relatives (six individuals' relationship to index case was unknown).

3.3. Cutaneous and uterine leiomyomata

Of 185 individuals from 55 families, 90 (48.6%) had confirmed cutaneous leiomyomata, with an age-related prevalence to age 75 yr of 78.0% (Table 1, Fig. 1A, and Supplementary Table 2).

Thirty-three females from 26 families (33/107 = 30.8%) had confirmed uterine leiomyomata, with an age-related prevalence of 42.1% by age 75 yr (Table 1 and Fig. 1B).

3.4. Renal lesions

Of 185 individuals, 23 (12.4%; 13 males and 10 females; 20 symptomatic presentations and three screen detected) individuals had confirmed (Cancer Registry/histological records) renal tumours (Supplementary

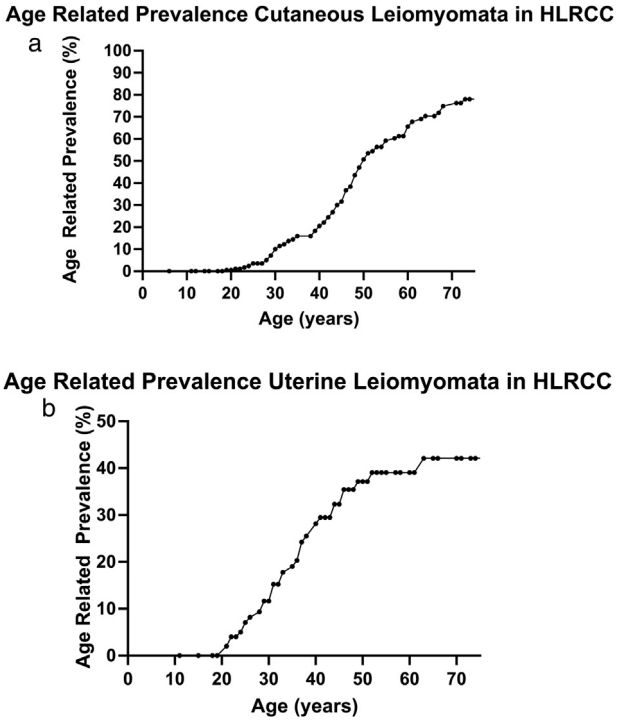

Fig. 1 – Age-related prevalence of cutaneous and uterine leiomyomata in HLRCC cohort (numbers of individuals at risk shown below graph). (A) Age-related prevalence of cutaneous leiomyomata in males and females. (B) Age-related prevalence of uterine leiomyomata in females. HLRCC = hereditary leiomyomatosis and renal cell cancer.

Table 3). An additional four cases of renal cancer reported by family history (all overseas) but unconfirmed were excluded.

Histological subtype of renal tumour was known in 18/23 cases and comprised seven clear cell renal cell carcinomas (CCRCCs), six papillary type 2 RCCs, three papillary RCCs (no further subclassification available), one collecting duct RCC, and one tumour showing an oncocytic cystic morphology. All were unifocal except for one papillary RCC that was multifocal.

3.5. *Symptomatic presentations of RCC*

Seven individuals presented with stage 4 disease, two with stage 3 disease, four with stage 2 disease, and one with stage 1 disease. Mean maximum diameter of RCC, where known, was 93.8 mm ($n = 5$, median = 88 mm, range = 70–136 mm, SEM = 11.89). Mean age of symptomatic presentation of RCC was 44.0 yr, significantly younger than both sporadic RCC ($p = <0.0001$) and familial non-VHL CCRCC ($p = 0.0080$), but similar to that associated with symptomatic presentation in VHL disease [11,12] (Fig. 2A and Table 2). Age-related prevalence of renal cancer was 20.8% by age 75 yr (Fig. 2B, and Supplementary Tables 2 and 4) and mean survival following diagnosis of symptomatic RCC was 38.1 mo (Fig. 2C and Table 2).

A prospective symptomatic RCC occurred in a 49-yr-old female who presented with a pT3a RCC (Fig. 3A) following

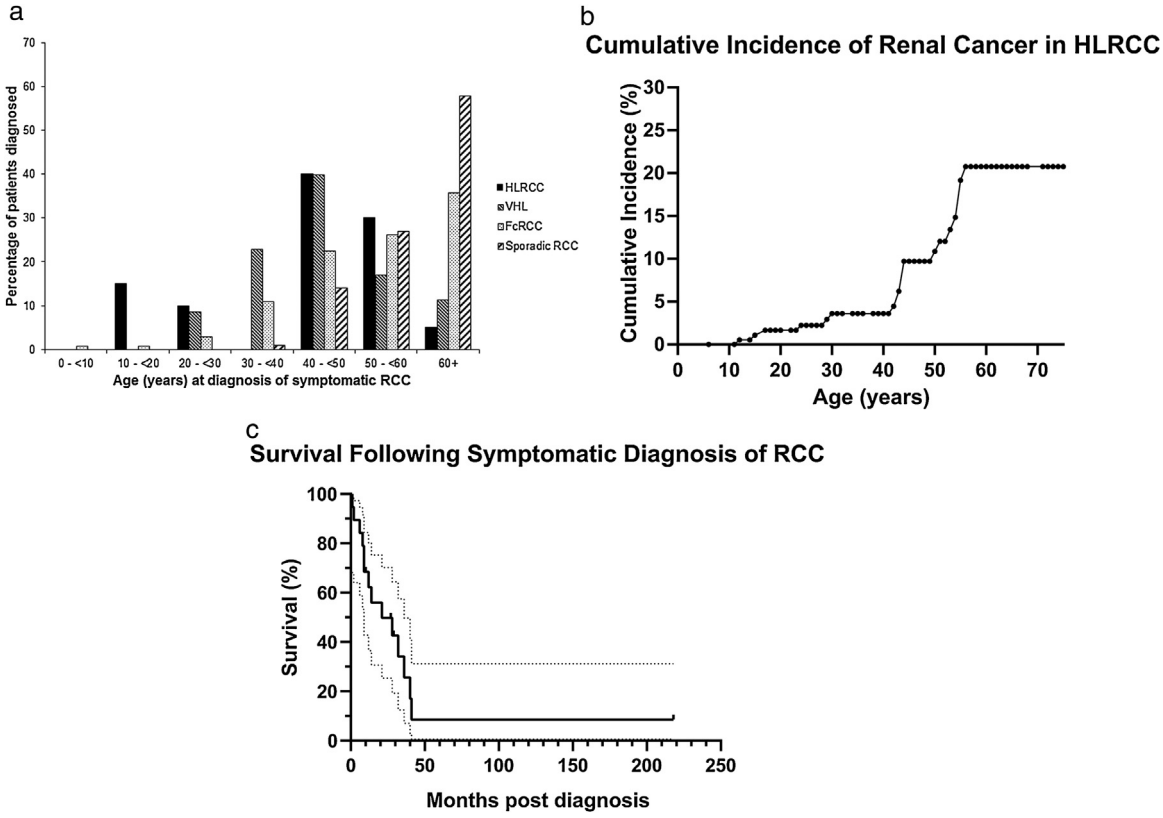

Fig. 2 – Clinical features of RCCs diagnosed in HLRCC cohort. (A) Histogram showing age at diagnosis of symptomatic presentation of RCC in 19 individuals within the HLRCC cohort as compared with VHL disease, FcRCC, and sporadic RCC [11,12]. (B) Age-related prevalence of RCC in HLRCC cohort. (C) Kaplan-Meier curve showing overall survival following symptomatic diagnosis of RCC in 19 individuals (solid line; 95% CIs indicated by dotted lines). CI = confidence interval; FcRCC = familial clear cell RCC; HLRCC = hereditary leiomyomatosis and renal cell cancer; RCC = renal cell carcinoma; VHL = von Hippel-Lindau.

Table 2 – Details of screen detected and symptomatic RCCs (excluding oncocytic-cystic lesion as not RCC) occurring within the HLRCC cohort.

Features of Symptomatic RCCs								
Age at diagnosis	Mean age diagnosis, yr (SEM)	N[a]	Range (yr)	Median (yr)	Mean age at diagnosis comparison (yr)			
					Sporadic RCC[b]	Symptomatic VHL disease[c]	Symptomatic FCRCC[d]	
All	44.0 (3.5)	19	14–79	44	61.0 $t = 4.6580$ $p \leq 0.0001$	46.2 $t = 0.6425$ $p = 0.5234$	53.2 $t = 2.6838$ $p = 0.0080$	
Index	41.1[e] (4.75)	8	17–54	43.5	–	–	–	
Nonindex	46.0[e] (4.98)	11	14–79	42	–	–	–	
Survival of symptomatic RCCs					Stage 1/2 disease		Stage 3/4 disease	
Mean survival, mo (SEM)		N*	95% CIs	Median survival (mo)	Mean survival, mo (SEM)	Number of cases	Mean survival, mo (SEM)	Number of cases
38.1 (15.7)		19	7.2–68.9	21.0	80.7[f] (16.6)	8	15.8[f] (3.9)	9 [g]
Screen-detected RCCs								
	Age at detection (yr)	Sex	Screening scan number	Imaging features	Surgery	Pathology	Stage	Status and censoring point
Individual #1	30	M	4	MRI: complex renal cyst with enhancement	Partial nephrectomy	Fuhrman grade 1 1 cm CCRCC	pT1a	Alive, 42 mo
Individual #2	43	F	1	MRI: complex cystic lesion upper pole CT: lesion suspicious for RCC	Partial nephrectomy	Fuhrman grade 3 4.4 cm CCRCC	pT1b	Alive, 18 mo
Individual #3 [h]	11	M	1	USS: renal mass CT: soft tissue mass lower pole right kidney	Total nephrectomy	Fuhrman grade 2 6.5 cm papillary RCC	pT1b	Alive, 101 mo

CCRCC = clear cell RCC; CI = confidence interval; CT = computed tomography; df = degree of freedom; F = female; FCRCC = familial clear cell RCC; HLRCC = hereditary leiomyomatosis and renal cell cancer; HR = hazard ratio; M = male; MRI = magnetic resonance imaging; RCC = renal cell carcinoma; SEM = standard error of the mean; USS = ultrasound scan; VHL = von Hippel-Lindau.

[a] Number of cases where age at diagnosis known.

[b] Maher et al [11].

[c] Maher et al [33].

[d] Woodward et al [12].

[e] Mean age comparison, $t = 0.68$, $p = 0.50$.

[f] Mean survival comparison: HR = 14.42, 95% CIs = 4.03–51.58, $\chi^2 = 12.6$, df = 1, $p = 0.0004$.

[g] All patients deceased by 36 mo after diagnosis.

[h] Alrashdi et al [9].

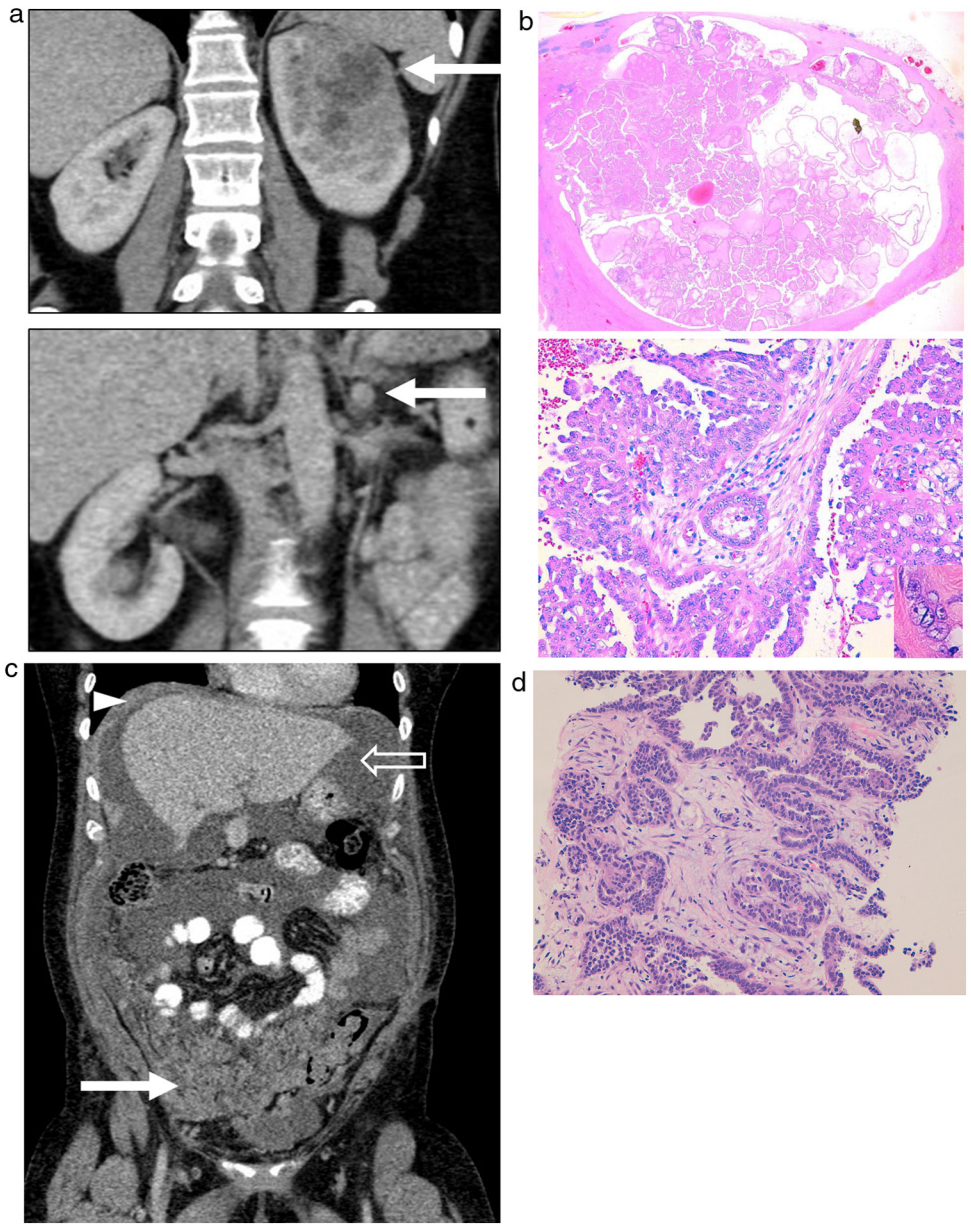

Fig. 3 – Prospective symptomatic presentation of RCC. (A) Contrast-enhanced coronal reformat CT at diagnosis showing a large complex mass at the upper pole of the left kidney, highly suggestive of an RCC (arrowed, upper panel) and a suspicious lymph node (arrowed, lower panel). (B) H&E histological stain showing dominant papillary architecture at low power (upper panel) with large prominent nucleoli at high power (lower panel 20×, inset 40×). (C) Coronal reformat image of contrast-enhanced CT 1 yr following the initial presentation and left radical nephrectomy. There is omental caking (solid arrow), ascites (unfilled arrow), and solid peritoneal masses (arrow head) in keeping with malignant peritoneal disease. (D) H&E histological stain (20×) of peritoneal biopsy demonstrating metastatic RCC with papillary architecture and large dominant nucleoli. CT = computed tomography; H&E = haematoxylin and eosin; RCC = renal cell carcinoma.

the diagnosis of HLRCC in a relative. Histological review following complete nephrectomy demonstrated a 70 × 50 × 60 mm Fuhrman grade 3 papillary RCC (no subtype given; Fig. 3B). Subsequent review in the genetics clinic noted the presence of cutaneous leiomyomata, a history of uterine fibroids, and the presence of a germline *FH* mutation (c.301C > T; p.Arg101*). Six months after nephrectomy, she developed liver metastases followed by peritoneal metastases and omental caking, and died 14 mo after the initial diagnosis (Fig. 3C and 3D).

3.6. *Renal imaging screening*

Eighty-one individuals with no personal history of renal tumours from 35 families with a diagnosis of HLRCC underwent screening imaging, and three presymptomatic RCCs were detected (Table 2 and Supplementary Table 5). Twenty-one different additional radiological findings (none required invasive investigation) were detected in 62 individuals, including 41 individuals with renal cysts (Supplementary Table 6).

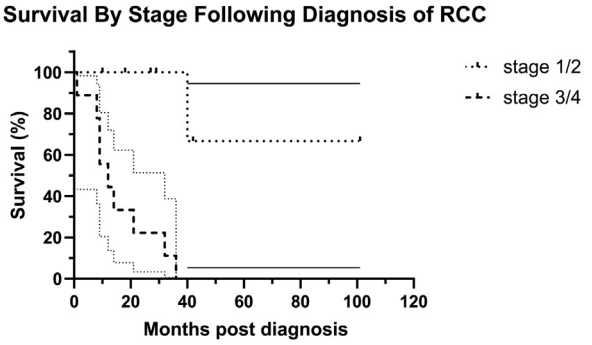

Fig. 4 – Survival (in months) after the diagnosis of HLRCC-associated RCC in this cohort following diagnosis at stage 1/2 disease (bold dotted line; 95% CIs indicated by solid lines) versus stage 3/4 disease (broken line; 95% CIs indicated by fine dotted lines). CI = confidence interval; HLRCC = hereditary leiomyomatosis and renal cell cancer; RCC = renal cell carcinoma.

3.7. RCC survival and stage

Considering both screen-detected and symptomatic RCC presentations, mean survival of individuals diagnosed with stage 3 or 4 RCC was significantly shorter at 15.8 mo (all nine of nine patients deceased at 36 mo) as compared with 80.7 mo for individuals diagnosed with stage 1 or 2 RCC (Fig. 4 and Table 2).

3.8. Other cancer diagnoses

Twelve individuals from eight families had confirmed diagnoses (medical records/Cancer Registry) of a total of 11 different non-RCC cancers, with three individuals having two cancer diagnoses (Supplementary Table 7).

3.9. Molecular genetic testing

In 68/69 families, an underlying *FH* variant was detected (one family declined testing). In the 68 families, 19 different novel *FH* variants were detected in 19 families (nine missense, four nonsense, two frameshift, one in-frame deletion-insertion, one frame-shift deletion-insertion, one in frame duplication, and one splice site; Supplementary Table 8). Although many of these *FH* variants had been detected in the families prior to the introduction of the American College of Medical Genetics and Genomics (ACMG) guidelines for variant interpretation into the UK [14,15], we undertook a retrospective ACMG classification of these 19 novel variants, finding 11 to be likely pathogenic and eight pathogenic.

For the remaining 49 families in which testing was undertaken, 21 non-novel *FH* variants were identified (12 missense, three nonsense, three frameshift, one in-frame duplication, one in-frame deletion, and one multiexon deletion) with 10/21 variants occurring in more than one family (Supplementary Table 9). Two of 21 variants occurring in three families, c.521C > G; p.Pro174Arg and c.1431_1433dupAAA; p.Lys477dup (including the family in our cohort with fumarase deficiency), had been described previously in recessive fumarase deficiency but not, to our knowledge, in the heterozygous state causing HLRCC.

4. Discussion

This study is the largest single clinical and molecular study of HLRCC to date, involving 185 individuals from 69 families (mean 2.68 cases/family). Compared with other recent studies with a lower mean number of cases per family (1.60) [3], our series has less ascertainment bias (probands: nonprobands = 1:2.25) and more detailed clinical information, and should provide more accurate estimates of tumour risks.

Whilst the penetrance for cutaneous and uterine leiomyomata is reported as being upward of 70% [3], our study found 48.1% of individuals to have cutaneous leiomyomata (cumulative incidence 51% by 50 yr and 76% by 71 yr) and 28% of females to have uterine leiomyomata (cumulative incidence 37% by 50 yr and 42% by 70 yr). This likely arises from both our strict inclusion criteria necessitating confirmed diagnoses and less than one-third of our cohort being accounted for by index cases. We cannot exclude that there may be under-reporting and underdiagnosis of these lesions; however, given their benign nature, it would be difficult to justify regular clinical follow-up and invasive imaging to attain exact affected/unaffected figures.

Of all individuals, 12.4% developed renal tumours. HLRCC was classically associated with papillary type 2 RCC [17]; however, more recently a wider spectrum of renal tumour histology has been described [4,7], which our data support. Many of our cases predate the 2016 classification of renal tumours, whereby HLRCC associated RCC is a distinct entity with characteristic histological appearance [6]. If it were possible to re-examine these cases, it would be intriguing to see whether they match this classification. Mean age at diagnosis of symptomatic RCC was similar to that reported previously [1,3] and in VHL disease [11]. However, whilst typically in familial RCC, there is a predisposition to the development of multifocal RCCs [18], patients in this cohort presenting with symptomatic RCCs typically had large unifocal tumours.

The propensity to develop large aggressive unifocal tumours in HLRCC has been recognised previously [4,17] and may have some bearing on the poor survival. Median survival of symptomatic RCC was only 14 mo, with all nine patients presenting with stage 3/4 disease having died at 36 mo after the diagnosis, similar to that reported previously [3].

Why these tumours are so aggressive is unclear but is recognised. Considering all RCCs with an undifferentiated histological appearance, those associated with FH deficiency have been shown to have worst clinical outcome [19]. Similarly, our data concur with that from The Cancer Genome Atlas where median survival for CpG island methylator phenotype RCCs, previously shown to be present in FH-deficient RCCs, is <1 yr [20,21].

Accumulation of fumarate has also been shown to be associated with multiple protumourigenic mechanisms

including inappropriate HIF pathway upregulation (pseudohypoxia) [22], increased expression of target genes of the NRF2 oncogenic transcription factor [23,24], epigenetic alterations (DNA and histone methylation) [25], promotion of epithelial to mesenchymal transition [26], and impaired homologous recombination DNA repair activity [27]. Elucidation of these dysregulated cellular pathways provides insights into potential therapeutic options for metastatic FH-deficient RCC such as inhibitors of downstream targets of the HIF activation pathway or PARP inhibitors.

Whilst targeted treatments are effective in improving survival in cancer medicine, cure is rarely achieved, and cancer prevention or early detection strategies are considered most likely to enable curative intervention [28]. Thus, renal surveillance imaging, preferably by MRI with 1–3 mm slices, similar to that in other familial RCC predisposition syndromes is recommended [1]. However, the efficacy of such surveillance has not, to our knowledge, been investigated previously. We demonstrated that annual renal surveillance imaging resulted in earlier-stage tumour detection. Whilst our numbers were too small to demonstrate a survival benefit in screened individuals, the three individuals with screen-detected RCCs were diagnosed with stage 1 disease. We also demonstrated a clear survival benefit for individuals diagnosed with stage1/2 versus stage 3/4 disease. Based on these findings, we support the previous consensus recommendation of annual renal imaging screening, by MRI, commencing at age 10 yr to enable early RCC detection in HLRCC [1].

Our data suggest that renal imaging screening in HLRCC is sensitive and specific. Three RCCs were diagnosed; there were no interval cancers, and none of the additional findings required invasive investigation.

Assuming a 21% RCC risk to age 70 yr and poor outcomes from symptomatic RCC presentation, our data suggest that as few as five patients with HLRCC would need to be screened to save one life (assuming early detection and treatment were curative). Whilst further data and a detailed health economic analysis are required to determine a definitive cost-benefit analysis for RCC screening in HLRCC, the available data suggest that the cost of a screening programme might be less than the cost of treating an individual with limited life expectancy presenting with advanced disease.

Whilst germline *FH* mutations have also been described in approximately 1% individuals presenting with phaeochromocytoma/paraganglioma (PPGL) only, and PPGL has been reported in 1% of HLRCC cases [3,29], none in this cohort developed a PPGL. Screening for PPGL in asymptomatic HLRCC patients is not routinely undertaken in the UK, which our data would support.

In almost one-third of our families, a novel *FH* variant was detected. Using ACMG guidance, we classified these variants as being either likely pathogenic or pathogenic. We found no evidence of any genotype-phenotype correlation in keeping with previous studies.

Of the previously described mutations, two (c.521C > G; p.Pro174Arg, c.1431_1433dupAAA;p.Lys477dup) have been described in fumarase deficiency rather than in HLRCC [8,30]. The first was identified in two unrelated individuals, one of whom presented with fumarase deficiency and paternal partial isodisomy of chromosome 1 [8]. The father had two skin lesions removed previously, but we were unable to clarify their nature.

We detected the *FH* variant c.1431_1433dupAAA;p.Lys477dup in two families with RCC only and no other features of HLRCC. This variant has previously been reported in fumarase deficiency [30] and an isolated case of ovarian cystadenoma [31], and is present in neither gnomAD (https://gnomad.broadinstitute.org/) nor ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Therefore, this may represent either a rare *FH* allele predisposing to RCC or, given its location outside of the known functional domains [32], a rare coincidental variant.

5. Conclusions

This is the single largest clinical and molecular study of HLRCC. Our data demonstrate that early diagnosis of HLRCC, with screening being offered to at-risk individuals, provides opportunities for disease prevention in this highly aggressive cancer predisposition syndrome.

Author contributions: Emma R. Woodward had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Maher, Woodward.
Acquisition of data: Forde, Lim, Alwan, Butland, Cleaver, Dixit, Hanson, Lalloo, Oliveira, Vialard, Wallis, Woodward.
Analysis and interpretation of data: Forde, Alwan, Burghel, Evans, Lalloo, Oliveira, Vialard, Wallis, Maher, Woodward.
Drafting of the manuscript: Forde, Alwan, Burghel, Evans, Lalloo, Oliveira, Maher, Woodward.
Critical revision of the manuscript for important intellectual content: Forde, Evans, Hanson, Lalloo, Oliveira, Maher, Woodward.
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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.euo.2019.11.002.

References

[1] Menko FH, Maher ER, Schmidt LS, et al. Hereditary leiomyomatosis and renal cell cancer (HLRCC): renal cancer risk, surveillance and treatment. Fam Cancer 2014;13:637–44.

[2] Tomlinson IP, Alam NA, Rowan AJ, et al. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat Genet 2002;30:406–10.

[3] Muller M, Ferlicot S, Guillaud-Bataille M, et al. Reassessing the clinical spectrum associated with hereditary leiomyomatosis and renal cell carcinoma syndrome in French FH mutation carriers. Clin Genet 2017;92:606–15.

[4] Merino MJ, Torres-Cabala C, Pinto P, Linehan WM. The morphologic spectrum of kidney tumors in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome. Am J Surg Pathol 2007;31:1578–85.

[5] Sanz-Ortega J, Vocke C, Stratton P, Linehan WM, Merino MJ. Morphologic and molecular characteristics of uterine leiomyomas in hereditary leiomyomatosis and renal cancer (HLRCC) syndrome. Am J Surg Pathol 2013;37:74–80.

[6] Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO classification of tumours of the urinary system and male genital organs—part A: renal, penile, and testicular tumours. Eur Urol 2016;70:93–105.

[7] Chen YB, Brannon AR, Toubaji A, et al. Hereditary leiomyomatosis and renal cell carcinoma syndrome-associated renal cancer: recognition of the syndrome by pathologic features and the utility of detecting aberrant succination by immunohistochemistry. Am J Surg Pathol 2014;38:627–37.

[8] Zeng WQ, Gao H, Brueton L, et al. Fumarase deficiency caused by homozygous P131R mutation and paternal partial isodisomy of chromosome 1. Am J Med Genet A 2006;140:1004–9.

[9] Alrashdi I, Levine S, Paterson J, et al. Hereditary leiomyomatosis and renal cell carcinoma: very early diagnosis of renal cancer in a paediatric patient. Fam Cancer 2010;9:239–43.

[10] Whitworth J, Smith PS, Martin JE, et al. Comprehensive cancer-predisposition gene testing in an adult multiple primary tumor series shows a broad range of deleterious variants and atypical tumor phenotypes. Am J Hum Genet 2018;103:3–18.

[11] Maher ER, Yates JR, Ferguson-Smith MA. Statistical analysis of the two stage mutation model in von Hippel-Lindau disease, and in sporadic cerebellar haemangioblastoma and renal cell carcinoma. J Med Genet 1990;27:311–4.

[12] Woodward ER, Ricketts C, Killick P, et al. Familial non-VHL clear cell (conventional) renal cell carcinoma: clinical features, segregation analysis, and mutation analysis of FLCN. Clin Cancer Res 2008;14:5925–30.

[13] Plon SE, Eccles DM, Easton D, et al. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat 2008;29:1282–91.

[14] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–24.

[15] Ellard S, Baple EL, Owens M, et al. ACGS best practice guidelines for variant classification. Association for Clinical Genetic Science http://www.acgs.uk.com/media/1059605/uk_practice_guidelines_for_variant_classification_2017_24_05_17.pdf.20172017

[16] AJCC. AJCC cancer staging manual. https://cancerstaging.org/references-tools/deskreferences/Documents/AJCC%207th%20Ed%20Cancer%20Staging%20Manual.pdf2010

[17] Launonen V, Vierimaa O, Kiuru M, et al. Inherited susceptibility to uterine leiomyomas and renal cell cancer. Proc Natl Acad Sci USA 2001;98:3387–92.

[18] Maher ER. Hereditary renal cell carcinoma syndromes: diagnosis, surveillance and management. World J Urol 2018;36:1891–8.

[19] Chen YB, Xu J, Skanderup AJ, et al. Molecular analysis of aggressive renal cell carcinoma with unclassified histology reveals distinct subsets. Nat Commun 2016;7:13131.

[20] Ricketts CJ, De Cubas AA, Fan H, et al. The Cancer Genome Atlas comprehensive molecular characterization of renal cell carcinoma. Cell Rep 2018;23:313–26.

[21] Cancer Genome Atlas Research Network. Comprehensive molecular characterization of papillary renal-cell carcinoma. New Engl J Med 2016;374:135–45.

[22] Pollard PJ, Briere JJ, Alam NA, et al. Accumulation of Krebs cycle intermediates and over-expression of HIF1α in tumours which result from germline FH and SDH mutations. Hum Mol Genet 2005;14:2231–9.

[23] Ooi A, Wong JC, Petillo D, et al. An antioxidant response phenotype shared between hereditary and sporadic type 2 papillary renal cell carcinoma. Cancer Cell 2011;20:511–23.

[24] Adam J, Hatipoglu E, O'Flaherty L, et al. Renal cyst formation in Fh1-deficient mice is independent of the Hif/Phd pathway: roles for fumarate in KEAP1 succination and Nrf2 signalling. Cancer Cell 2011;20:524–37.

[25] Xiao M, Yang H, Xu W, et al. Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. Genes Dev 2012;26:1326–38.

[26] Sciacovelli M, Gonçalves E, Johnson TI, et al. Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition. Nature 2016;537:544–7.

[27] Sulkowski PL, Sundaram RK, Oeck S, et al. Krebs-cycle-deficient hereditary cancer syndromes are defined by defects in homologous-recombination DNA repair. Nat Genet 2018;50:1086–92.

[28] Independent Cancer, Taskforce. Achieving world-class cancer outcomes: a strategy for England 2015–2020. https://www.england.nhs.uk/cancer/strategy/2015

[29] Clark GR, Sciacovelli M, Gaude E, et al. Germline FH mutations presenting with pheochromocytoma. J Clin Endocrinol Metab 2014;99:E2046–50.

[30] Alam NA, Rowan AJ, Wortham NC, et al. Genetic and functional analyses of FH mutations in multiple cutaneous and uterine leiomyomatosis, hereditary leiomyomatosis and renal cancer, and fumarate hydratase deficiency. Hum Mol Genet 2003;12:1241–1252.

[31] Ylisaukko-oja SK, Cybulski C, Lehtonen R, et al. Germline fumarate hydratase mutations in patients with ovarian mucinous cystadenoma. Eur J Hum Genet 2006;14:880–3.

[32] Bulku A, Weaver TM, Berkmen MB. Biochemical characterization of two clinically-relevant human fumarase variants defective for oligomerization. Open Biochem J 2018;12:1–15.

[33] Maher ER, Yates JRW, Harries R, et al. Clinical features and natural history of von Hippel-Lindau disease. Q J Med 1990;77:1151–63.